Portable Sensors: Testing the Next Generation of Quality Assurance Devices for the Soybean Industry

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ABSTRACT

Soybean varieties have been genetically modified for increased oleic acid content to achieve a healthier and more stable oil product. With these new genetically modified organisms (GMO), it is economically important that the oil and protein content of the soybean are not reduced and that rapid methods of differentiating these new varieties and their components are found. The use of infrared (IR) spectroscopy, a form of vibrational spectroscopy, is an attractive method for the food industry due to its ability to rapidly qualify and quantify characteristics of many foods. The objective was to develop a rapid non-targeted screening approach to authenticate GMO high oleic versus non-GMO conventional soybeans by combining IR spectroscopy with pattern recognition analysis. Soybean samples (n=60) were kindly provided by DuPont Pioneer including equal numbers of GMO high oleic and non-GMO conventional varieties. Soybeans were homogenized and their composition was characterized by reference methods for fat analysis (Soxhlet, AOAC #945.16), protein analysis (Dumas, ICC Standard No. 167), and fatty acid profile (Gas Chromatography, AOAC #996.06). Spectra was collected with a portable Fourier transform infrared (FTIR) spectrometer and analyzed by Soft Independent Modeling of Class Analogy (SIMCA) and Partial Least Square Regression (PLSR) to develop classification and quantitative (fat and protein) algorithms. The GMO soybeans contained higher oleic acid content (54%), lower levels of polyunsaturated (50%) and saturated fats (4.3%), and similar levels of protein (~38%) and fat (~16%) content compared to their non-GMO counterpart. Pattern recognition analysis showed there was significant difference between the IR spectra of the GMO and non-GMO soybeans with a class distance of 6.4 and that the genetic modification had made a phenotypical difference in the chemical profile. Regression analysis associated the IR signal with both fat and protein components. Regression models were generated for non-destructive and rapid determination of oil (R_{CV}=0.98 and SECV=0.19) and protein (R_{CV}=0.98 and SECV=0.06) levels. The results showed that portable spectroscopy devices have potential to be used for a rapid, in-field, and non-destructive method to identify different varieties of soybeans and screen for specific traits, making this a great alternative to time-consuming analytical methods.

Keywords: High oleic oil, Soybeans, Plenish®, Infrared Spectroscopy, Portable Devices

INTRODUCTION

Soybeans [*Glycine max* (L.) Merr.] are legumes whose value largely depend on the oil and protein content (Rotundo & Westgate, 2009; Medic, Atkinson, & Hurburgh, 2014). Soybeans account for approximately 29% of the world's vegetable oil consumption and 71% of the world's protein meal consumption (American Soybean Association, 2016a,b).

Many characteristics of the oil are determined by the fatty acid make-up, including its nutritional value and oxidative stability. Thus, genetic engineering has produced new soybean varieties with increased oleic fatty acid, a monounsaturated fat (Cahoon, 2003; DuPont Pioneer, 2014; Medic et al., 2014). High oleic soybean oil can generally be considered a healthier oil due to it having lower saturated fat and greater unsaturated fat with the former typically associated with negative impact on health and the latter with a more positive impact (Lunn and Theobald, 2006). These oils have a higher oxidative stability and longer shelf life compared to conventional soybean oil due to the lower amount of polyunsaturated fatty acids (Cahoon, 2003). Hydrogenation is not needed for this higher stability and trans-fat is not created which is important for the food industry with the banning of partially hydrogenated oils (Food and Drug Administration, 2015; Kim et al., 2015). With these new genetically modified organism (GMO) soybean varieties, it is economically important that the oil and protein content are not reduced by the modification as this could result in overall decreased profit (Graef et al., 2009).

Monitoring incoming soybeans as raw ingredients is of importance for food companies for both product quality and verification (Rodriguez-Saona & Allendorf, 2011). Rapid monitoring of the quantity of oil and protein content in soybeans prior to processing can ensure quality of finished products and continued accuracy to the nutrition label (Nielsen, 2014). It is also of importance due to the big price different of different types of oils. With increased regulation of GMO food ingredients, rapid methods for differentiating GMO and non-GMO soybean varieties is important for authentication of ingredients received, specifically for non-GMO verified products (Ahmed, 2002). Traditional methods for measuring the fat and protein and differentiating varieties can be time-consuming and laborious. For example, the use of Soxhlet (AOAC #945.16) for determining total fat amount takes at least 5 hours and profiling the fatty acids using Gas Chromatography (AOAC #996.0) with fatty acid methyl ester derivatization takes ~3 hours (Kinton, 2019; Firestone & Yurawecz, 2016). Both of these have complicated sample preparation and need skilled workers for good results.

The use of infrared (IR) spectroscopy, a form of vibrational spectroscopy, is an attractive method for ingredient monitoring for the food industry due to its ability to rapidly qualify and quantify characteristics of many foods (Rodriguez-Saona and Allendorf, 2011; Wehling, 2014). IR spectroscopy involves measuring the absorption of infrared light and a change in dipole moment from functional groups associated with food components such as fatty acids and proteins (Wehling, 2014). The resulting spectrum collected with this method is unique, much like a fingerprint, making IR spectroscopy an ideal technique for identification and characterization of ingredients during quality control (Scotter, 1997). IR spectroscopy is of advantage over traditional methods because it can result in time and cost saving due to these devices being simple and rapid to use, needing only small sample sizes and minimal preparation, and minimizing solvent use (Rodriguez-Saona and Allendorf, 2011; Sivam, Sun-Waterhouse, Perera, & Waterhouse, 2013). For this technique, portable devices are becoming more available to the industry that parallel the accuracy of benchtop equipment, making this approach more suitable for in field analysis. However, per food, specific models need to be created for the monitoring of food items with these devices (Wehling, 2014).

PROBLEM IDENTIFICATION AND JUSTIFICATION

In this study, two aims were addressed, the use of IR spectroscopy as a quality control tool for soybean varieties and to compare the oil and protein composition in specific varieties of Plenish® GMO high oleic and non-GMO conventional soybeans.

With the growing production of new specialty soybean oils such as high oleic oils and increased regulation of GMO food ingredients it is becoming very important for companies to be able to monitor incoming soybeans (Ahmed, 2002). However, traditional methods can be time-consuming, causing slowdowns in the production of products, specifically at ingredient receiving. Traditional methods can also be labor intensive and take skilled labor, which consumes the time of workers that could be spent elsewhere or could result in inaccurate results with workers that are not as experienced. Thus, finding rapid and simple methods for identification is of importance for food companies to continue efficient production. Portable IR spectroscopy have potential to fit this need.

In addition, Plenish® GMO high oleic soybeans and non-GMO conventional soybeans will be compared to identify how beneficial the genetic engineering was for this specific high

oleic soybean variety for oil and protein content. This study aims to identify what is gained from growing this variety of high oleic soybeans over conventional soybeans in terms of oil and protein content. Both Zhang et al. (2014) and Graef et al. (2009) found that the high oleic soybean variety they studied did not have a significantly different total oil amount than conventional soybeans. In contrast, Kim et al. (2015) found the oil content to be lower than conventional soybeans. Each study found that the protein content was not significantly different. A decrease in the amount of oil or protein is not wanted as this can decrease the overall economic value received from the soybeans.

Creating a soybean variety that has an improved oil composition while still maintaining oil and protein content could be important for several reasons including some of the following. Genetically modifying soybeans for higher oleic acid composition could be one way of increasing the availability of high oleic vegetable oil which is in limited supply and restricts its use in the market (Wilson, 2012). High oleic oil is a potential solution for replacing partially hydrogenated oils in products. Soybean protein could be increasingly important to meet protein and sustainability demands in the future (Day, 2013).

OBJECTIVES

Specific objectives were to:

1. Develop a rapid non-targeted screening approach to authenticate Plenish® GMO high oleic soybeans versus non-GMO conventional soybeans by combining portable IR spectroscopy with analytical reference methods and pattern recognition analysis.
2. Analyze and compare the oil and protein content and fatty acid profile of these soybean varieties from DuPont Pioneer, looking specifically at their Plenish® product.

MATERIALS AND METHODS

Soybean samples were kindly donated by DuPont Pioneer's Plenish® Division that included 30 Plenish® high oleic and 30 conventional soybeans varieties. Plenish® is a GMO high oleic soybean engineered by Dupont Pioneer. Soybeans were homogenized in a Waring blender under liquid nitrogen, producing fine particles and high surface area that allowed improving extraction of the target compounds, and used for all reference methods and IR spectroscopy analysis. All tests were done in duplicates.

REFERENCE METHODS

Fat and Protein Analysis

Fat analysis to measure fat content was done by using a Soxhlet gravimetric method (AOAC #945.39) (Kinton, 2016). The percolating process was run for a total of 6 hours for a total of 240 cycles/refluxes. The extracted oil was stored at refrigeration temperature (4°C) until used for gas chromatography analysis. Protein analysis was done by the Dumas method reported in ICC Standard No. 167 from the International Association for Cereal Science and Technology (ICC, 2000). A factor of 6.25 was multiplied to the % nitrogen in order to calculate total % protein in the soybeans samples.

Fatty Acid Profile

Determination of fatty acid profile in extracted soybean oil was done with a fatty acid methyl ester (FAME) derivatization for gas chromatography (AOAC #996.0) (Firestone & Yurawecz, 2016). Samples were analyzed on an Agilent HP-6890 GC equipped with a flame ionization detector (FID) and HP G1513A auto-sampler (Agilent, Santa Clara, CA). Separation of the components was done using an HP-88 100m x 0.25mm x 0.2um column (Agilent, Santa Clara, CA) using helium as the carrier gas. The injection volume was 0.2µL in the split mode (50:1). The oven conditions were 125°C to 220°C (5°C/min) hold for 15 minutes. Injector temperature was 220°C and the detector temperature was 250°C. The identification of fatty acids was carried out by comparing the retention times with an A-68C fatty acid standard (Nu-Check Prep Inc., Elysian, MN). Concentration of each fatty acid was calculated based on the percentage area under the peak.

INFRARED SPECTROSCOPY

Infrared spectral data was collected on portable Cary 630 Fourier transform-infrared spectrometer (Agilent Technologies Inc., Santa Clara, CA) equipped with a Permaglow™ (ceramic) source, single-bounce attenuated total reflectance (ATR) diamond crystal interface, and a thermoelectrically cooled dTGS detector (**Figure 1**). Soybean samples (~0.1g) were placed directly on the surface of the diamond ATR crystal and spectrum was collected in the 4,000–700 cm^{-1} region at 4 cm^{-1} resolution by pressing the powder sample onto the crystal using a pressure clamp. A background was taken prior to each sample run and the data was collected by co-adding 64 scans.

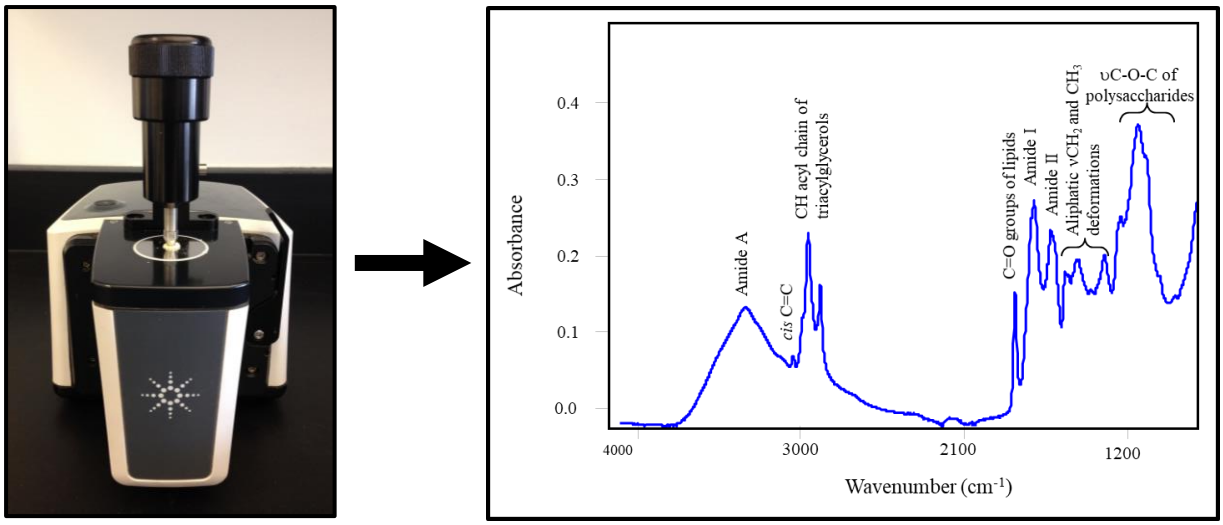

Figure 1 Shows the representative IR spectra obtained from Plenish® high oleic soybean samples taken with the Portable Cary 630.

MULTIVARIATE ANALYSIS

All spectral data was analyzed using multivariate statistical analysis software (Pirouette version 4.5, Infometrix, Bothell, WA). The spectra were imported into the software from the instruments as GRAMS (.spc) files. Spectra pre-treatments included normalization to reduce multiplicative scaling effects and smoothing (35-point window) to reduce noise.

Soft Independent Modeling of Class Analogy

Classification analysis between high oleic soybeans and conventional soybean samples was done by soft independent modeling of class analogy (SIMCA). SIMCA is a supervised pattern recognition classification technique that requires a previous knowledge about the category membership of samples, and thus, forty-eight total samples of the Plenish® high oleic and conventional soybeans were used as the calibration set to train the IR device. The model performance was evaluated by the use of a validation set comparing predictions with true categories. Twelve randomly selected samples of Plenish® high oleic and conventional soybeans were used as an independent validation set. All replicates belonging to the same soybeans sample were either used only in the calibration or validation set.

Partial Least Squares Regression

Quantitative models were generated with partial least squares regression (PLSR) using the IR spectra and reference values measured for oil and protein content of the calibration set of soybeans samples. PLSR reduces the dimensions contained in thousands of IR data points into a

few factors to explain variations in both the dependent variables and the spectral domain and helps to eliminate data noise (Zhao, 2015). Similarly to formerly stated, the calibration set was made up of forty-eight samples of Plenish® high oleic and conventional soybeans and the performance was validated with twelve independent samples not included in the calibration model. Samples containing abnormal residual pattern and high leverage were reanalyzed and if deemed outliers excluded if necessary. The model was evaluated based on standard error of cross validation (SECV), correlation coefficient of cross-validation (R_{CV}), standard error of prediction (SEP), and correlation coefficient of prediction (R_V).

RESULTS AND DISCUSSION

Soybean Protein, Oil, and Fatty Acid Profile

Plenish® GMO high oleic soybeans (protein=38.2 ± 0.15, fat=15.5 ± 1.2) and non-GMO conventional soybeans (protein=37.5 ± 0.22, fat=16.2 ± 1.4) showed similar levels of protein and oil (p=0.06 and 0.71, respectively), indicating the genetic modification of the Plenish® high oleic varieties did not significantly affect protein and fat content. Furthermore, our data shows the Plenish® high oleic and conventional soybean varieties fall within the parameters of the USDA Soybean Export Assessment Data for oil and protein content. Average oil and protein levels ranged from 15.5-16.2% and 37.5-38.2%, respectively, which are within the range reported by the USDA (USDA, 2011).

The fatty acid compositions of the Plenish® high oleic and conventional soybeans were analyzed and investigated (**Table 1**). There was no significant difference in steric acid (p=0.62) but a significant difference (p<0.05) in palmitic, oleic, linoleic and linolenic acids. The fatty acid values obtained from our study were comparable to Dupont Pioneer's optimal profile of fatty acids for Plenish® high oleic soybeans (Dupont Pioneer, 2017).

The Plenish® high oleic soybeans contained different compositional breakdown of mono- and poly-unsaturated fats compared to conventional soybeans. Monounsaturated fats (MUFAs) accounted for oleic acid which was 54% higher in the Plenish® high oleic soybean varieties. The increase in MUFAs are desirable because these fatty acids not only improve shelf life but also reduces the need for hydrogenation, a process which generates unwanted *trans*-fat which has been linked to many health problems in humans (Mozaffarian & Clarke, 2009; Kim et al., 2015). Polyunsaturated fatty acids comprised of the linoleic and linolenic acids showed a reduction of

50% in the Plenish® high oleic soybeans varieties which can aid in increasing the shelf life due to the reduction of the amount of double bonds (Cahoon, 2003). The Plenish® high oleic soybeans also contained 4.3% less saturated fats (made of the palmitic and stearic acids) which is desirable due to excess saturated fat in the diet leading to a negative impact on health (Lunn and Theobald, 2006).

Table 1 Fatty acid profile between Plenish® high oleic (n=30) and conventional (n=30) soybeans analyzed for all the parameters measured. Values were run in duplicate (n=120).

Trait*	High Oleic[a]	Conventional[b]
Palmitic Acid	6.8 ± 0.9	11.8 ± 1.2
Steric Acid	4.5 ± 0.5	3.8 ± 0.9
Oleic Acid	75.8 ± 2.1	21.9 ± 1.5
Linoleic Acid	7.5 ± 0.6	53.8 ± 2.3
Linolenic Acid	3.2 ± 1.2	7.1 ± 1.6

[a] Data are means of all Plenish® high oleic soybean samples ± standard deviations; [b] Data are means of all conventional soybean samples ± standard deviations; *Fatty acid composition (% of total lipid)

Classification

SIMCA analysis was used on the soybean spectra to statistically discern and profile differences between data sets of the Plenish® high oleic and conventional soybean varieties. **Figure 2(A)** shows the SIMCA plot of the two classes obtained from the IR spectra. Two well-separated classes were identified showing interclass distance (ICD) of 6.4 which is significant to differentiate between two classes (Vogt & Knutsen, 1985). In **Figure 2(B)**, the SIMCA discriminating power showed the variables (wavenumbers) responsible for the clustering between the Plenish® high oleic and conventional soybean varieties. The infrared signal centered at 3060-2770 cm^{-1} and 1763-850 cm^{-1} were found to be very important in this ability to differentiate between Plenish® high oleic and conventional soybeans varieties. These show that the IR spectroscopy had the ability to assign each soybean variety to its correct class and thus, identifying soybean varieties that accumulate oleic acid. This good ability to separate the two classes can be related to the ability of IR to distinguish the C=C *cis* band which is correlated with unsaturated fatty acid, prevalent in high oleic soybeans. The IR region between 3040 and 2850 cm^{-1} also provides information about the C–H groups for asymmetrical and symmetrical

stretching's of CH_2 and CH_3 which characterizes fatty acid chains, and 1654 cm^{-1} related to N–H vibrational stretching for proteins (Carbonaro & Nucara, 2010).

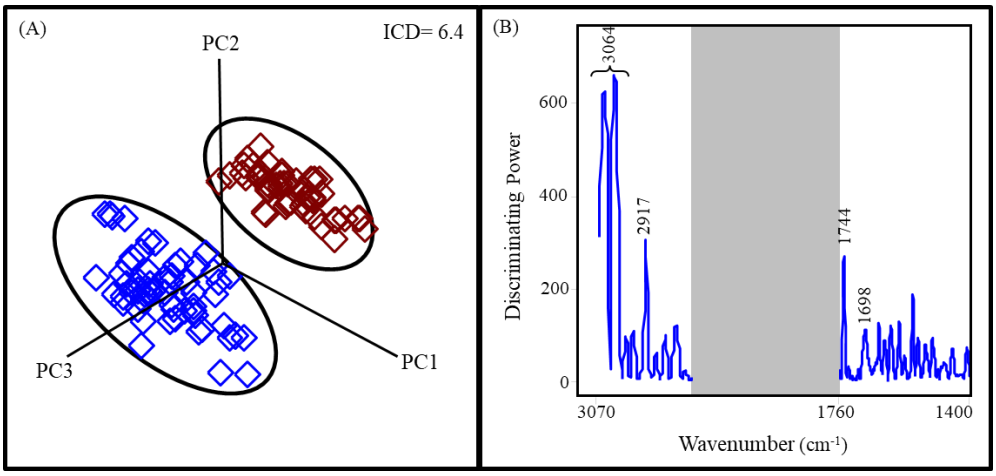

Figure 2 SIMCA plot classifying Plenish® high oleic (blue) and conventional (red) soybeans with a significant (A) interclass distance of 6.4 and certain (B) discriminating power.

Regression

PLSR was the main algorithm used to obtain the prediction models to estimate the protein and oil content of soybeans by using the IR spectra. The correlations between the IR estimated value and reference value for both oil and protein content are illustrated in **Figure 3 (A)** and **(B)**, respectively**.** The plots showed the excellent agreement between the actual and predicted values for the calibration and validation sets. Performance statistics of these PLSR models are shown in **Table 2**.

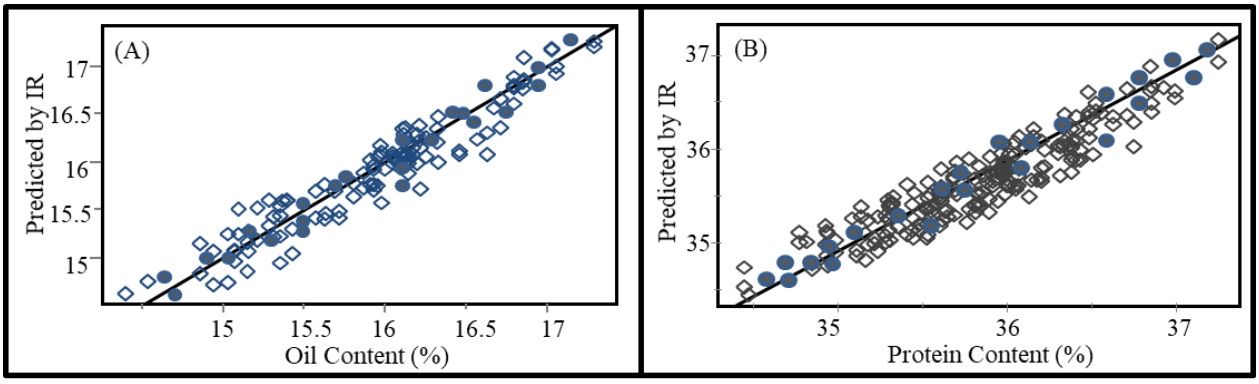

Figure 3 PLSR plots for oil (A) and protein (B) content in Plenish® high oleic and conventional soybeans by IR spectra results. (◊ and ● represent samples in calibration and validation groups, respectively.)

Table 2 Performance of prediction models developed for the IR device.

Parameter	Factors[a]	SECV[b]	SEP[c]	R_{CV}[d]	R_V[e]
Protein (%)	4	0.06	0.09	0.98	0.97
Oil (%)	3	0.19	0.20	0.98	0.97

[a]Factors: Set of factors that account for most of the response's variation; [b]SECV: Standard error of cross-validation; [c]SEP: Standard error of prediction; [d]R_{CV}: Correlation coefficient of cross-validation; [e]R_V: Correlation coefficient of prediction

As seen in **Table 2**, there were 3 and 4 factors that accounted for most of the variation for the response for oil and protein, respectively. The IR device was able to accurately predict both the protein and oil content shown by the high R_{CV} (0.98) for both regression models. The regression model for the protein content was found to be more precise than the oil content shown by the SECV (protein=0.06, oil=0.19), but overall good precision was seen for both. The similar values of the R_V (0.97) to the R_{CV} and SEP (protein=0.09, oil=0.20) to the SECV showed that the protein and oil regression models were robust and were not changed by adding additional independent samples, which was tested in this study with the validation sets. The IR selected effective regions that were used to achieve this accuracy and precision were 870 cm^{-1} to 1700 cm^{-1} for protein and 3065 to 2770 cm^{-1} and 1765 to 1700 cm^{-1} for the oil which were consistent with double bonds. Thus, IR was shown to be effective in predicting both the protein and oil content in soybeans both rapidly (~1 min) and simply once the models were created.

CONCLUSIONS

The results revealed that the Plenish® GMO high oleic soybean varieties did contain a fatty acid profile with higher oleic acid content and this modification did not significantly decrease the total oil and protein content compared with the non-GMO counterpart. Thus, Plenish® high oleic soybeans have the potential to be more economically beneficial for growers and food companies and provide higher levels of monounsaturated fats for consumers over the conventional soybeans. Furthermore, the portable IR spectroscopy device was shown to have the ability to rapidly differentiate between the two varieties and accurately predict the oil and protein levels compared to AOAC and ICC reference methods with minimal sample preparation and skilled knowledge needed for running the IR device. Therefore, IR spectroscopy devices have potential to be used for a rapid, in-field, and simple method to identify different varieties of

soybeans and screen for specific traits, making this a great alternative to time-consuming analytical reference methods.

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